Asignment

Information Assets

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UV / VISIBLE SPECTROPHOTOMETER

1. Basic Principles of UV-Vis Measurement

1.1 The electromagnetic spectrum: Ultraviolet (UV) and visible radiation are a small part of the electromagnetic spectrum, which includes other forms of radiation such as radio, infrared (IR), cosmic, and X rays.

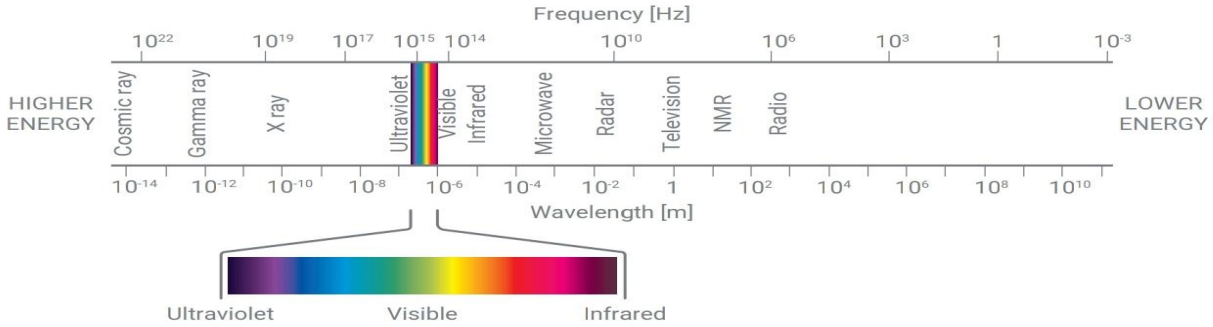
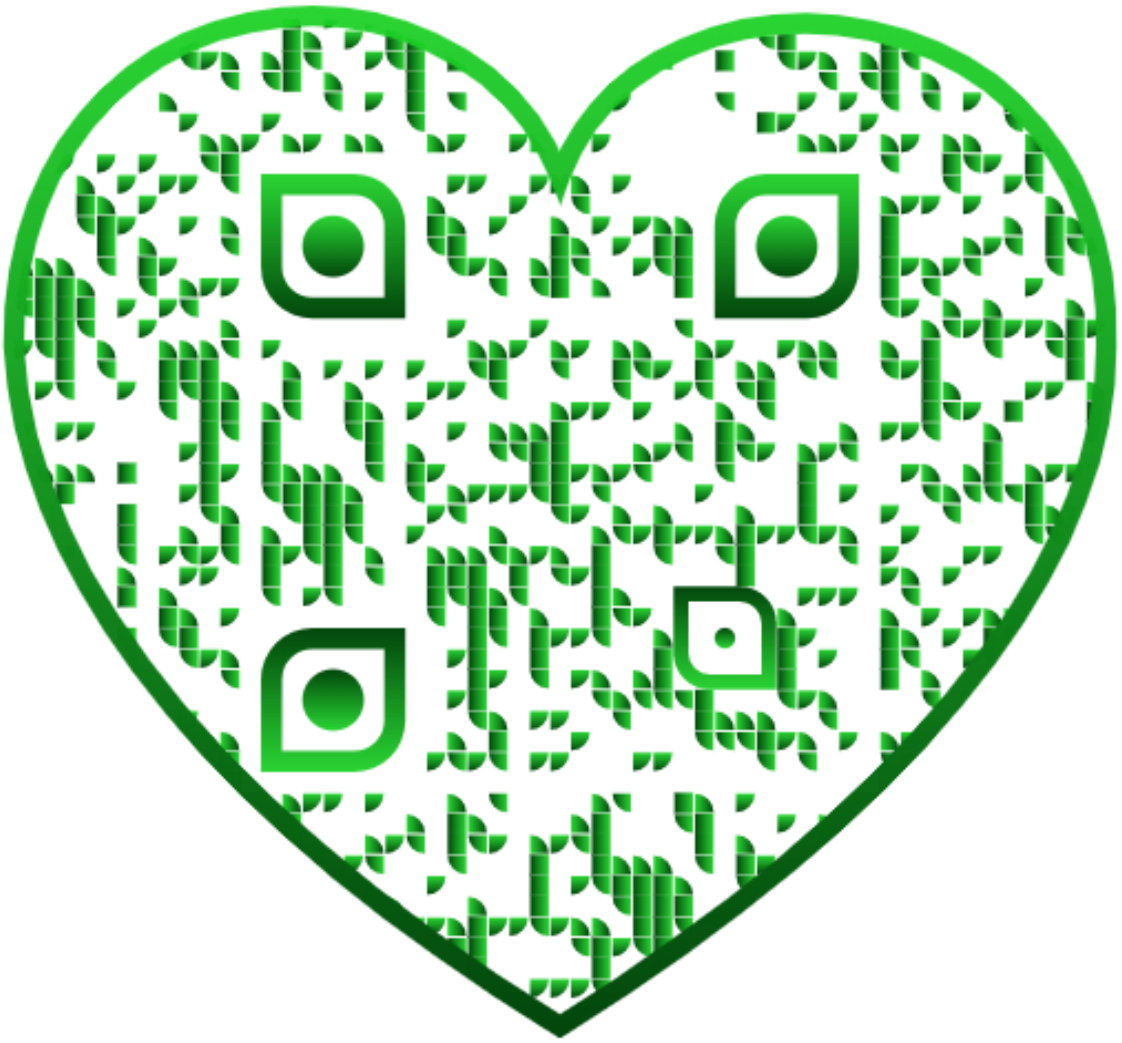


Figure 1. The electromagnetic spectrum, with the visible light section expanded.

The energy associated with electromagnetic radiation is defined as:

$E = h\nu$ where E is energy (in joules), h is Planck's constant (6.62 × 10-34 Js), and v is frequency (in seconds).



spectroscopy : is the study of the introduction between the matter and electromagnetic radiation (EMR) , In another meaning Spectroscopy allows the study of how matter interacts with or emits electromagnetic radiation.

There are different types of spectroscopy, depending on the wavelength range that is being measured. UV-Vis spectroscopy uses the ultraviolet and visible regions of the electromagnetic spectrum. Infrared spectroscopy uses the lower energy infrared part of the spectrum.

1.2 Wavelength and frequency Electromagnetic radiation can be considered a combination of alternating electric and magnetic fields that travel through space in a wave motion. Because radiation acts as a wave, it can be classified in terms of eitherr wavelength or frequency, which are related by the following equation:

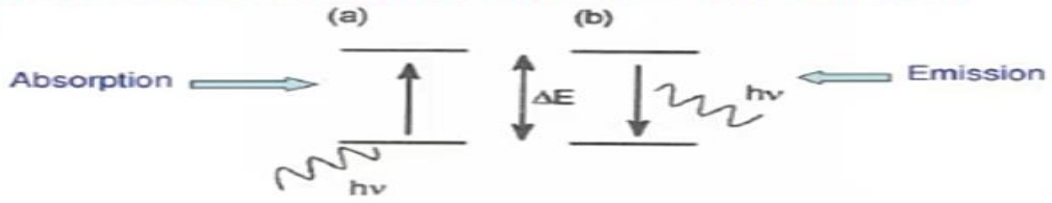
$\nu = c/\lambda$ where ν is frequency (in seconds), c is the speed of light (3×108 ms-1), and λ is wavelength (in meters).

In UV-Vis spectroscopy, wavelength is usually expressed in nanometers (1 nm = 10-9m).

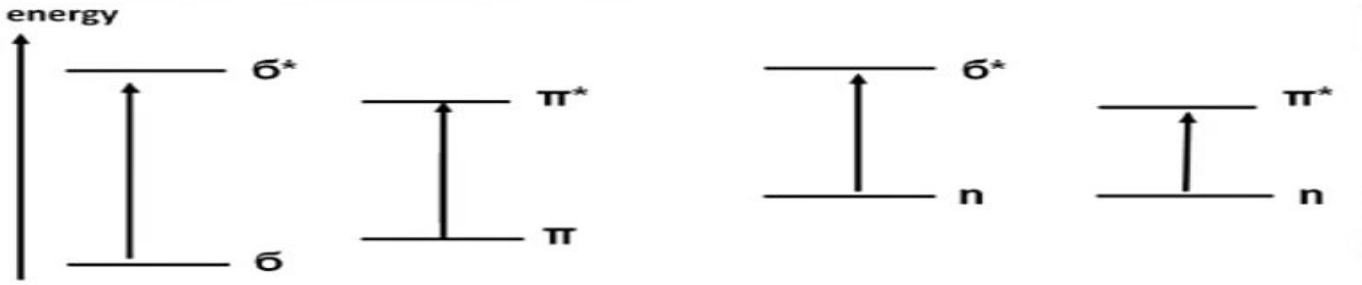
It follows from the equations that radiation with shorter wavelength has higher energy, and, for UV-Vis spectroscopy, the low (short) wavelength UV light has the highest energy. Sometimes, this energy may be sufficient to cause unwanted photochemical reactions when measuring samples that are photosensitive.

2. Effect of Conjugation on λmax and Color measurement.

- UV/Vis spectroscopy provides information about compounds with conjugated double bonds
- It can be also used for qualitative and quantitative determination of organic pharmaceuticals.
- UV-VIS region of EMR : 200 - 800nm.
- When U.V or visible radiation passes through compound .., electrons in ground state absorb certain quantity of energy and transmitted to excited state



- possible electronic transitions :



Only organic compounds with π electrons can produce UV/Vis spectra.

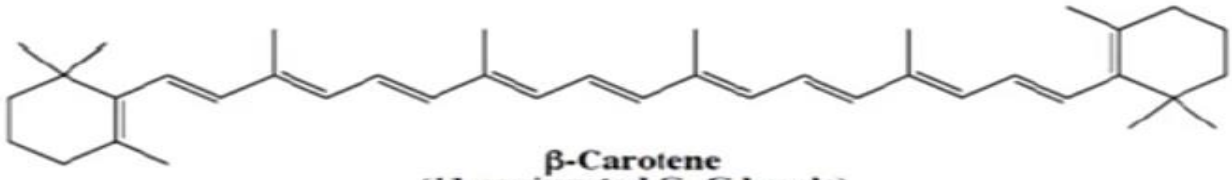
σ-σ* transition → Δ E is high, thus the transition requires short λ
ex: In $CH_3—CH_3$ σ-σ* transition requires λ max = 135 nm
N.B. Any transition that requires λ < 200 nm is out of scope (need vacuum UV)
π-π* transition: → Δ E is lower than σ-σ*, thus the transition requires a longer λ for example, π-π* $CH_2 = CH_2$ (λ max = 175 nm)
n-π* transition: → Δ E is lower than π-π*, thus the transition requires a longer λ, for example, n-π* of $(CH_3)_2C=O$ (λ max = 279 nm).
n-σ* transition: n-σ* of H-O-H requires EMR with λ max = 180 nm

The most important electronic transitions are π-π* and n-π*, why?

Because both of them can absorb in the UV/VIS region of EMR radiation.

↑conjugation ↑ absorption λ max

β-Carotene , contains 11 conjugated double bonds , λ max of its π-π* = 465 nm.



β-Carotene
(11 conjugated C=C bonds)
(orange color)

Why are some organic compounds colored while others aren't? b-Carotene, the pigment in carrots, is purple-orange, for instance, while cholesterol is colorless. The answer involves both the chemical structures of colored molecules and the way we perceive light. The visible region of the electromagnetic spectrum is adjacent to the ultra-violet region, extending from approximately 400 to 800 nm. Colored compounds have such elaborate systems of conjugation that their "UV" absorptions extend into the visible region. b-Carotene, for example, has 11 double bonds in conjugation, and its absorption occurs at λmax 455 nm.

"White" light from the sun or from a lamp consists of all wavelengths in the visible region. When white light strikes b-carotene, the wavelengths from 400 to 500 nm (blue) are absorbed while all other wavelengths are transmitted and can reach our eyes. We therefore see the white light with the blue removed, which we perceive as a yellow-orange color for b-carotene. Conjugation is crucial not only for the colors we see in organic molecules but also for the light-sensitive molecules on which our visual system is based. The key substance for vision is dietary b-carotene, which is converted to vitamin A by

enzymes in the liver, oxidized to an aldehyde called 11-trans-retinal, and then isomerized by a change in geometry of the C11–C12 double bond to produce 11-cis-retinal.

♦Color measurement: Color is an important property of a material. The color of matter is related to its absorptivity or reflectivity of specific wavelengths of light. The human eye sees the complementary color to that which is absorbed, ...

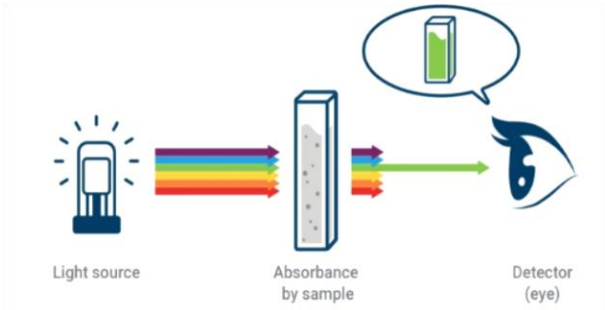


Figure 37. Transmission and color. Much like the spectrophotometer detector, our eye sees the light transmitted through or bounced off a surface. We perceive this as the color of the object.

. Measuring color

UV-Vis spectrophotometers are used to measure the color of liquids and solids. By measuring the visible light that is transmitted through a solution or material, or the light that is reflected from a surface, the color of the liquid or material can be calculated. A series of three numbers is used to indicate the coordinates of the measured color in a 'color space'.

Figure 38. The wavelengths of light associated with different colors (left) and the color of absorbed light and the associated complementary color the human eye sees (right).

3. Instrumental design Components.

The key components of a spectrophotometer are:

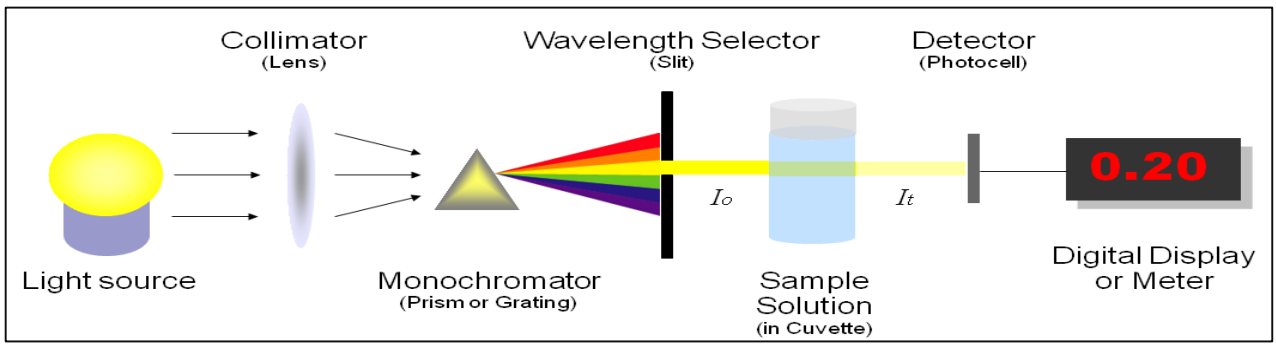
– A light source that generates a broadband of electromagnetic radiation across the UV-visible spectrum

– A dispersion device separates the broadband radiation into wavelengths

– A sample area, where the light passes through or reflects off a sample.

– One or more detectors to measure the intensity of the reflected or transmitted radiation

Other optical components, such as lenses, mirrors, or fiber-optics, relay light through the instrument.



4. Overview of Common UV-Vis Applications.

1. Identification—spectra and structure

2. Confirmation of identity

3. Quantifying a molecule

Beer's law

If 100 photons of light enter a cuvette and only 50 emerge from the other side, the transmittance is 0.5, or 50 %. If these 50 photons then pass through an identical cuvette, only 25 will emerge, and so forth Lambert (1760) generally is credited with the first mathematical expression of this effect, although it now appears that Bouguer first stated it in 1729.

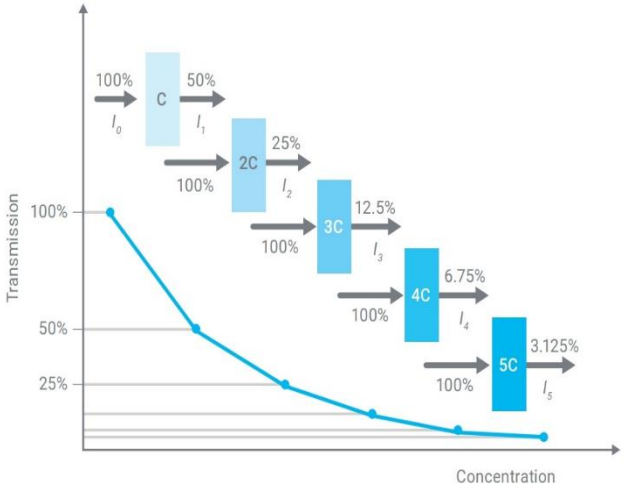


Figure 32. Transmittance and concentration—Beer's law

The equation is: T= I/Io = e–kb Where: Io is the incident intensity, I is the transmitted intensity e is the base of natural logarithms , k is a constant, b is the path length (usually in centimeters). Beer's law is identical to Bouguer's law, except that it is stated in terms of concentration. The amount of light absorbed is proportional to the number of absorbing molecules through which the light passes. Figure is shows a plot of transmittance against concentration.

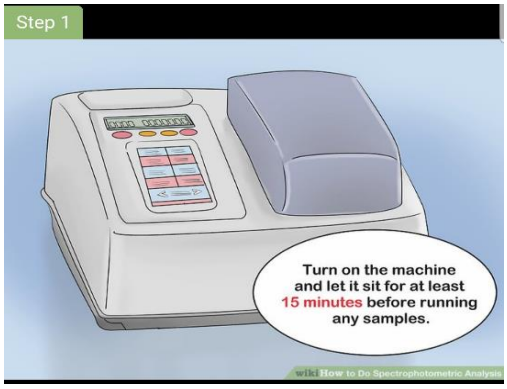
♦How Does a Modern UV-Vis Spectrophotometer Work?

Spectrophotometry is an experimental technique that is used to measure the concentration of solutes in a specific solution by calculating the amount of light absorbed by those solutes. This technique is powerful because certain compounds will absorb different wavelengths of light at different intensities. By analyzing the light that passes through the solution, you can identify particular dissolved substances in solution and how concentrated those substances are.

A spectrophotometer is the device used to analyze solutions in a laboratory research setting.

first [1] Preparing the Samples.

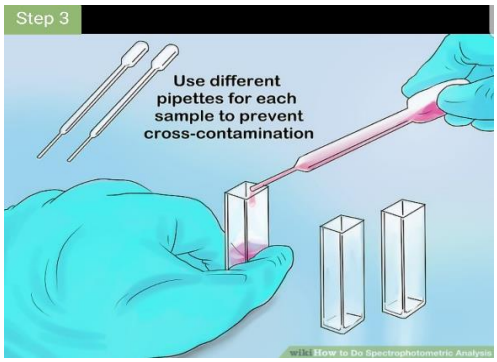
1. Turn on the spectrophotometer. Most spectrophotometers need to warm up before they can give an accurate reading. Turn on the machine and let it sit for at least 15 minutes before running any samples. Use the warm-up time to prepare your samples.



2. Clean the cuvettes or test tubes. If you are doing a lab for school, you may be using disposable test tubes that don't need to be cleaned. If you are using cuvettes or reusable test tubes, make sure they are properly cleaned before use. Rinse each cuvette thoroughly with deionized water.

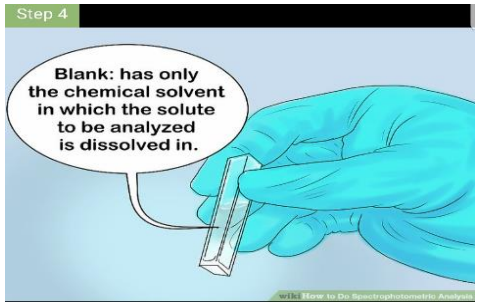
. Take care with cuvettes as they can be quite expensive, particularly if they are made from glass or quartz. Quartz cuvettes are designed for use in UV-visible spectrophotometry. When handling the cuvette, avoid touching the sides the light will pass through (generally, the clear sides of the container). If you accidentally touch these sides, wipe the cuvette down with a kimwipe (which are formulated to prevent scratching the glass)

3. Load the proper volume of the sample into the cuvette. Some cuvettes have a maximum volume of 1 milliliter (mL) while test tubes may have a maximum volume of 5 mL. As long as the laser producing the light is passing through the liquid and not an empty part of the container, you will get an accurate reading.



. If you are using a pipette to load your samples, use a new tip for each sample to prevent cross-contamination.

4. Prepare a control solution. Known as a blank, the control solution has only the chemical solvent in which the solute to be analyzed is dissolved in. For example, if you had salt dissolved in water, your blank would be just water. If you dye the water red, the blank must also contain red water. The blank is the same volume as the solution to be analyzed and kept in the same kind of container.



5. Wipe the outside of the cuvette. Before placing the cuvette into the spectrophotometer you want to make sure it is as clean as possible to avoid interference from dirt or dust particles. Using a lint free cloth, remove any water droplets or dust that may be on the outside of the cuvette.

Second [2] Running the Experiment.

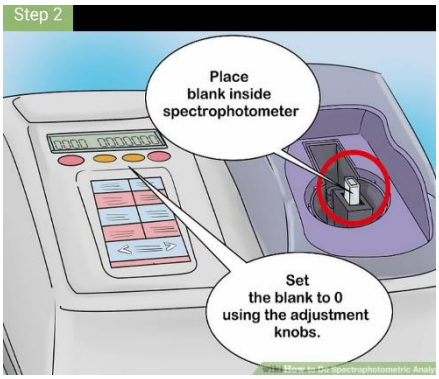
1. Choose and set the wavelength of light to analyze the sample with.

Use a single wavelength of light (monochromatic color) to make the testing more effective. The color of the light chosen should be one known to be absorbed by one of the chemicals thought to be in the test solute. Set the desired wavelength according to the specifications of your spectrophotometer.

. Because the sample will reflect all light of the same color as it appears, the experimental wavelength will always be a different color than that of the sample.

. Objects appear as certain colors because they reflect light of particular wavelengths and absorb all other colors. Grass is green because the chlorophyll in it reflects green light and absorbs everything else.

2. Calibrate the machine with the blank. Place the blank into the cuvette holder and shut the lid. On an analog spectrophotometer, there will be a screen with a needle that moves based on the intensity of light detection. When the blank is in, you should see the needle move to the right. Record this value in case you need it for later. With the blank still in the machine, move the needle to zero using the adjustment knob.

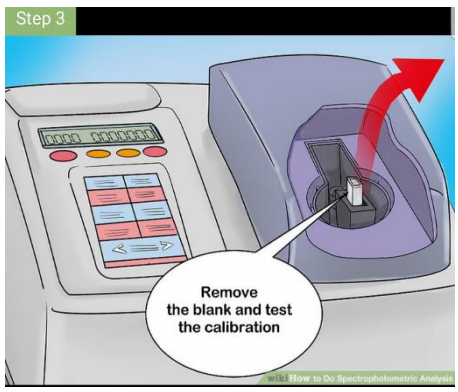


. Digital spectrophotometers can be calibrated in the same way, they will just have a digital readout. Set the blank to 0 using the adjustment knobs.

. When you remove the blank, the calibration will still be in place. When measuring the rest of your samples, the absorbance from the blank will automatically be subtracted out.

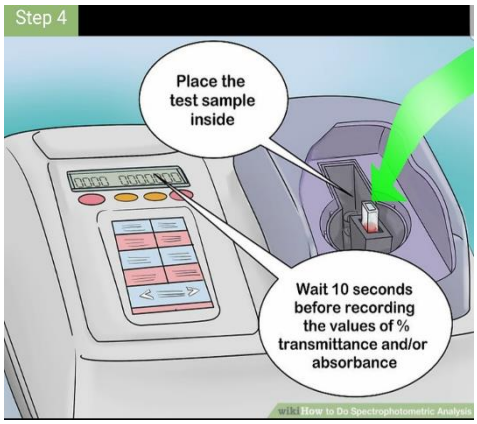
. Be sure to use a single blank per session so that each sample is calibrated to the same blank. For instance, if you blank the spectrophotometer, then analyze only some of samples and blank it again, the remaining samples would be inaccurate. You would need to start over.

3. Remove the blank and test the calibration. With the blank removed the needle should stay at 0 (zero) or the digital readout should continue to read 0. Place the blank back into the machine and ensure the needle or readout doesn't change. If the machine is properly calibrated with your blank, everything should stay at 0.



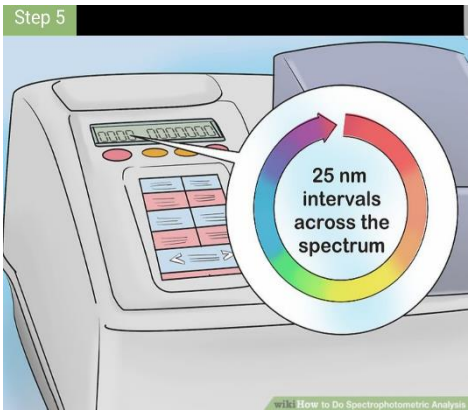
. If the needle or readout is not 0, repeat the calibration steps with the blank.
. If you continue to have problems, seek assistance or have the machine looked at for maintenance.

4. Measure the absorbance of your experimental sample. Remove the blank and place the experimental sample into the machine. Slide the cuvette into the designated groove and ensure it stands upright. Wait about 10 seconds until the needle is steady or until the digital numbers stop changing. Record the values of % transmittance and/or absorbance.


Step 4
Place the test sample inside
Wait 10 seconds before recording the values of % transmittance and/or absorbance
wikiHow to Do Spectrophotometric Analysis

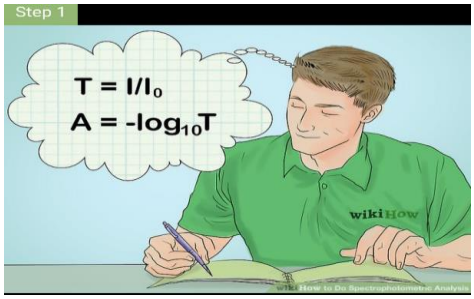
. The absorbance is also known as the optical density (OD).
. The more light that is transmitted, the less light the sample absorbs. Generally, you want to record the absorbance values which will usually be given as a decimal, for example, 0.43.
. If you get an outlying result (such as 0.900 when the rest are around 0.400), dilute the sample and measure the absorbance again.
. Repeat the reading for each individual sample at least 3 times and average them together. This ensures a more accurate readout.

5. Repeat the test with successive wavelengths of light. Your sample may have multiple unknown compounds that will vary in their absorbance depending on wavelength. To eliminate uncertainty, repeat your readings at 25 nm intervals across the spectrum. This will allow you to detect other chemicals suspected to be in the solute.



Third [3] Analyzing the Absorbance Data

1. Calculate the transmittance and absorbance of the sample. Transmittance is how much of the light that passed through the sample reached the spectrophotometer. Absorbance is how much of the light has been absorbed by one of the chemicals in the solute. Many modern spectrophotometers have an output of transmittance and absorbance, but if you recorded intensity, you can calculate these values.

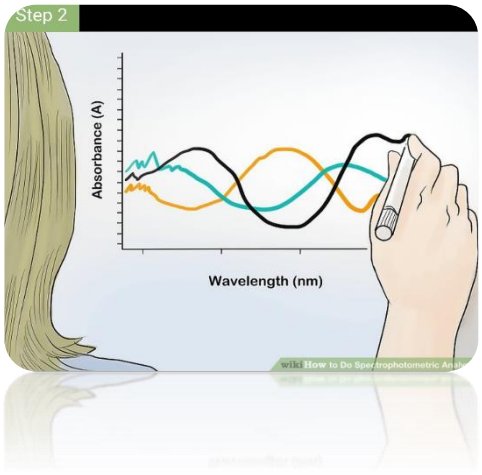


. The transmittance (T) is found by dividing the intensity of the light that passed through the sample solution with the amount that passed through the blank.

It is normally expressed as a decimal or percentage. T = I/I0 where I is the intensity of the sample and I0 is the intensity of the blank.

. The absorbance (A) is expressed as the negative of the base-10 logarithm (exponent) of the transmittance value; A = -log10T. For a T value of 0.1, the value of A is 1 (0.1 is 10 to the -1 power), meaning 10% of the light is transmitted and 90% is absorbed. For a T value of 0.01, the value of A is 2 (0.01 is 10 to the -2 power), meaning 1% of the light is transmitted.

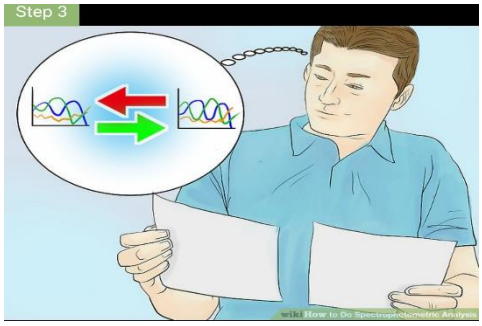
2. Plot the absorbance values versus the wavelengths on a graph. The absorbance value is plotted on the vertical y-axis against the wavelength of light used for a given test plotted on the horizontal x-axis. Plotting the maximum absorbance values for each wavelength of light tested, produces the sample's absorbance spectrum and identifies the compounds making up the test substance and their proportions.



. An absorbance spectrum usually has peaks at certain wavelengths that can allow you to identify specific compounds.

3. Compare your absorbance spectrum plot to known plots of specific compounds.

Compounds have unique absorbance spectrum and will always produce a peak at the same wavelength every time they are measured. By comparing your plots of unknown compounds to those of known compounds, you can identify the solutes that compose your solution.



. You can also use this method to identify contaminants in your sample. If you are expecting 1 clear peak at a specific wavelength and you get 2 peaks at separate wavelengths, you know something is not right in your sample.

The End,,,

References:

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2. The Basics of UV-Vis Spectrophotometry for © Agilent Technologies, Inc. 2021 Published in the USA, July 23, 2021 5980-1397EN.
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